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BRIEF REPORT

A Novel Missense Mutation in Human TTF-2 (FKHL15) Gene Associated with Congenital Hypothyroidism But Not Athyreosis


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Background: Thyroid dysgenesis is the most frequent cause of congenital hypothyroidism (CH), and its genetic basis is largely unknown. Hitherto, two mutations in the human thyroid transcription factor 2 (TTF-2) gene have been described in unrelated cases of CH with cleft palate, spiky hair, variable choanal atresia, and complete thyroid agenesis. Here, we describe a novel TTF-2 mutation in a female child resulting in syndromic CH in the absence of thyroid agenesis.

Results: The index case is homozygous for an arginine to cysteine mutation (R102C) of a highly conserved residue within the forkhead, DNA binding domain of TTF-2. Her consanguineous, heterozygous parents are unaffected, and the mutation was not detected in 100 control chromosomes. Consonant with its location, the R102C mutant TTF-2 protein showed loss of DNA binding and was transcriptionally inactive. CH in the proposita was associated with cleft palate, spiky hair, and bilateral choanal atresia. However, radiological studies showed the presence of thyroid tissue in a eutopic location.

Conclusion: Our findings indicate that human thyroid development can occur despite loss of TTF-2 function and suggest that TTF-2 gene defects should also be considered in cases of syndromic CH without total athyreosis. (J Clin Endocrinol Metab 91: 4183–4187, 2006)

CONGENITAL HYPOTHYROIDISM (CH), the most common neonatal endocrine disorder affecting one in 3000–4000 infants, is caused by either defects in hormone synthesis (dyshormonogenesis) or, most frequently (85% of cases), defective thyroid development (dysgenesis) (1). Whereas dyshormonogenetic causes of CH are associated with either goiter or a normal-sized thyroid gland, the spectrum of dysgenetic disorders includes thyroid ectopy, hypoplasia, and agenesis (2). Data from knockout mouse have demonstrated the role of several genes in thyroid organogenesis, and accordingly, mutations in PAX8, TSH receptor, and thyroid transcription factor genes, TTF-1 (NKX2–1) and TTF-2, have been reported in rare CH cases (3).

Thyroid transcription factor 2 (TTF-2) is a member of the forkhead/winged helix-domain protein family, many of which are key regulators of embryonic development (4). The human TTF-2 gene (also known as FKHL15 or FOXE1) is expressed continuously in the thyroid from early development and consists of a single exon encoding a 42-kDa protein of 367 amino acids. TTF-2 regulates the transcription of target genes such as thyroglobulin and thyroid peroxidase (TPO) by binding to specific regulatory DNA sequences within their promoters via its forkhead DNA binding domain (5–7).

Homozygous TTF-2 null mice exhibit cleft palate and thyroid malformation consisting of either thyroid agenesis or ectopic thyroid development (8). Homozygous, human TTF-2 mutations result in a syndromic form of dysgenetic CH (Bamforth-Lazarus syndrome), whose phenotype includes thyroid agenesis, cleft palate, and spiky hair, with or without choanal atresia and bifid epiglottis, depending on the severity of the mutation (9–11). Here we describe a child, born to consanguineous parents of Turkish origin, presenting with CH, bilateral choanal atresia, cleft palate, and spiky hair. The patient was homozygous for a novel missense mutation (R102C) within the forkhead domain of the TTF-2 protein. Unlike the TTF-2 mutations described previously, radiological examination of this patient revealed the presence of thyroid tissue in an eutopic location.

Subjects and Methods

Mutation screening

With informed consent, genomic DNA was isolated from whole blood and the entire coding exon of TTF-2 (accession no. NM_004473) was sequenced from the index case and first-degree relatives using previously described methods (11). The creation of a new AluNI re-
striction site by the mutation allowed screening of 100 control chromo-
somes using AliN1 (MBI Fermentas) digestion (4 h at 37 C).

Radiological studies

The presence of eupotic thyroid tissue was evaluated using 7.5 MHz
ultrasonography (at birth and 4 yr) and gadolinium-enhanced computed
tomography (CT) examination at age 9 yr.

Functional studies

DNA binding of wild-type and mutant TTF-2 was assessed by EMSA.
Wild-type TTF-2 DNA was amplified by PCR using genomic DNA from
a normal control and cloned as a BamHI-XhoI fragment into the pCDNA3
eukaryotic expression vector (Invitrogen, Carlsbad, CA). R102C mutant
TTF-2 was generated by site-directed mutagenesis of the wild-type
TTF-2 template using a standard protocol (Quickchange; Stratagene, La
Jolla, CA). All constructs were verified by direct sequencing. Equal
amounts of 35S-methionine-labeled wild-type or mutant TTF-2 proteins
were generated by in vitro translation (TNT; Promega, Madison, WI),
verified by SDS-PAGE analysis, and incubated for 30 min at room
temperature with a 125I-labeled oligonucleotide duplex corresponding to
the TTF-2 binding site in the thyroglobulin promoter (5'-GAGGGAGT-
TCCTGTGACTAGCAGAGAAAAC-AAAGTGAGCCAC-3') (12), in
buffer (20 mm HEPES, 10% glycerol, 2 mm dithiothreitol, 150 mm KCl,
PH 7.8) in the presence of 1 µg poly (dl-dc). Protein-DNA complexes
were resolved on a 6% polyacrylamide gel.

The transcriptional properties of wild-type and mutant TTF-2 were
tested by transient cotransfection in 293 EBNA cells (ECACC no.
85120002) grown in DMEM supplemented with 10% fetal bovine serum
and 1% penicillin, streptomycin, and fungizone (GIBCO-BRL, Carlsbad,
CA). A luciferase reporter gene (hTPO-LUC) was constructed by cloning
a 416-bp fragment (35–50) of the human TPO promoter (13) into
pA3LUC (14). Twenty-four-well plates were transfected with 500 ng
reporter gene, 10 ng TTF-1 expression vector, 100 ng Bos
gal, and 1–10
ng TTF-2 expression vector using the calcium phosphate method. After
36–48 h, cells were harvested, and luciferase and β-galactosidase assays
were performed with normalization of luciferase values to β-galactosi-
dase activity, as described previously (14).

Results

Clinical features

The proband, a 3900-g female infant, was born to consan-
guineous parents by normal vaginal delivery at 40 wk ges-
tation after an uncomplicated pregnancy. Postnatal exami-
nation revealed the patient to be hypotonic, hypoactive,
and areflexic with cleft palate, spiky hairs, and
bilateral choanal atresia, subsequently confirmed by para-
nasal sinus tomography. Meconium staining and perinatal
respiratory distress prompted admission to neonatal inten-
sive care for ventilatory support. Immediately after birth,
the patient’s total serum T4 level was 0.758 µg/dl [normal range
(NR), 6.1–14.9 µg/dl], and TSH was greater than 100
mIU/ml (NR, 1.7–9.1 mIU/ml). L-T4 replacement therapy
was started, and the baby was discharged at age 2 months.

Follow-up surveillance at age 19 months showed no neu-
rological abnormalities, and thyroid function tests on L-T4
therapy revealed TSH 0.57 mIU/ml, total T4 (TT4) 1.64 ng/ml
(NR, 0.8–2.0 ng/ml), total T3 (FT3) 11.3 µg/dl, free T3 (fT3)
3.94 pg/ml (NR, 2–4.4 pg/ml), free T4 (fT4) 1.85 ng/dl (NR,
0.9–1.7 ng/dl), and thyroglobulin less than 0.2 ng/ml (age-
related NR, 1–110 ng/ml). Reassessment at age 9 yr, after
withdrawal of L-T4 for 7 wk, showed profound biochemical
hypothyroidism (TT4 <1 µg/dl; TSH <0.4 ng/ml; TSH, 357
mIU/ml; fT4 <1 pg/ml; and fT4 <0.3 ng/dl). 131I scanning
showed negligible uptake, and serum thyroglobulin was 0.1
ng/ml (age-related NR, 2–65 ng/ml). The proband’s mother
(TT4 5.82 µg/dl; TSH, 2.33 mIU/ml), father (TT4 9.3 µg/dl;
TSH, 1.42 mIU/ml) and older male sibling (TT4 7.26 µg/dl;
TSH, 3.61 mIU/ml) are biochemically euthyroid with no congenital anomalies.

Radiological investigations

Neck ultrasonography performed at birth and age 4 re-
vealed heterogeneous, hyperechoic tissue in the paratracheal
region corresponding to a eupotic thyroid location. Recent
CT examination confirmed solid tissue in this location (Fig.
1A) consisting of right and left lobes and isthmus [anterior-
posterior diameters 1.96, 2.02, and 0.48 cm, respectively (nor-
mal diameter, <1.5 cm)], which failed to enhance with con-
trast, unlike normal, functioning thyroid tissue (Fig. 1B).

Mutation detection

Direct sequencing of the coding exon of TTF-2 gene revealed
the proband to be homozygous for a single nucleotide
substitution (CGC to TGC) at position 304, corresponding to
an arginine to cysteine mutation at codon 102 (R102C) within
the forkhead, DNA binding domain of the predicted protein
sequence. Both parents were heterozygous for the mutation,
consistent with an autosomal recessive mode of inheritance.

Consonant with the location of the R102C substitution
within the forkhead, DNA binding domain of TTF-2 and the
conservation of this residue in FOX genes from different
species or humans (Fig. 1, C and D), EMSAs showed negli-
gible binding of the R102C mutant to the human TPO promoter (13)
to pA3LUC (14). Twenty-four-well plates were transfected with 500 ng
reporter gene, 10 ng TTF-1 expression vector, 100 ng Bos
gal, and 1–10
ng TTF-2 expression vector using the calcium phosphate method. After
36–48 h, cells were harvested, and luciferase and β-galactosidase assays
were performed with normalization of luciferase values to β-galactosi-
dase activity, as described previously (14).

Functional characterization

Consonant with the location of the R102C substitution
within the forkhead, DNA binding domain of TTF-2 and the
conservation of this residue in FOX proteins from different
species or humans (Fig. 1, C and D), EMSAs showed negli-
gible binding of the R102C mutant TTF-2 protein to a known
TTF-2 response element (Fig. 2B).

The TPO gene promoter is known to be activated by TTF-2
(15), and cotransfection of wild-type TTF-2 augmented the
activity of a human TPO reporter gene in a dose-dependent
manner, whereas the R102C mutant TTF-2 mediated negli-
gible transcriptional activation, analogous to empty expres-
sion vector (Fig. 2).

The complete lack of DNA binding and transcriptional
activity of the R102C mutant are more analogous to the
properties of the A65V TTF-2 mutant described previously
(10) than the partial preservation of function noted with the
S57N mutant (11).

Discussion

We describe a child with CH associated with bilateral
choanal atresia, cleft palate, and spiky hair who is homozy-
gous for a novel, missense mutation (R102C) within the for-
head DNA binding domain of TTF-2. Both parents were
heterozygous for the mutation but euthyroid with no con-
genital anomalies, and the mutation was absent in 100 control
chromosomes. The arginine residue at codon 102 is highly
conserved in the forkhead domain family of proteins
(Fig. 1, C and D), and mutations of the equivalent amino acid
in other forkhead proteins [e.g. FOXC1 (R127H), FOXC2
(R121H), and FOXP2 (R553H)] have been described. Conso-

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nant with this, *in vitro* studies indicate that the mutation is highly deleterious, with the R102C mutant protein exhibiting negligible DNA binding and transcriptional activity. Interestingly, thyroid ultrasonography and CT examination of the proband indicated thyroid tissue in a eutopic location, although biochemical measurements and radioisotope scanning show that it is nonfunctional.

Our case represents the third recorded example of a loss-of-function mutation in the human TTF-2 gene, with the two previously described mutations (A65V and S57N) also being located within its forkhead, DNA-binding domain (10, 11). The A65V mutation was identified in a nonconsanguineous Welsh family with two male siblings exhibiting CH, cleft palate, choanal atresia, and bifid epiglottis together with spiky hair, whereas the S57N mutation was reported in two male siblings of a consanguineous Tunisian kindred exhibiting CH and cleft palate alone. With both of these TTF-2 mutation cases, 123I scanning and ultrasonography showed complete athyreosis (10, 11). In contrast, although the third case we report here shares some of these features, including severe CH and extrathyroidal anomalies, imaging indicates the presence of thyroid tissue in a eutopic location. However, severe biochemical hypothyroidism at birth and also following T4 withdrawal, together with absent 131I uptake and very low serum thyroglobulin levels, indicates that the function of such glandular tissue is markedly compromised.

Mouse models support a critical role for TTF-2 in thyroid and palate organogenesis. Expression of TTF-2, together with the transcription factors TTF-1 and PAX-8, has been demonstrated from the onset of formation of the thyroid primordium (embryonic d 8–8.5), continuing throughout the migration of the thyroid diverticulum (4, 16, 17). TTF-2 is also expressed in craniopharyngeal ectoderm involved in palate formation and Rathke’s pouch in mouse embryos (4) and in the outer follicular hair sheath in humans (18). Targeted disruption of the murine *Titf2* locus results in homozygous null mice with cleft palate and either complete thyroid agenesis or ectopic sublingual gland development (8).
murine phenotypes were seen with equal frequency and may reflect different developmental manifestations of the disorder. Thus, similar to the murine context, our human proband illustrates that thyroid morphogenesis can occur in the absence of TTF-2, albeit with migration of thyroid gland tissue to a eutopic location.

Mutations in TTF-2 and a TTF-1/NKX2.1 mutation (19) are the only known genetic causes of thyroid agenesis. However, involvement of TTF-2 accounts for only a small minority of CH cases, being a strong consideration only in those with cleft palate, which is an infrequent association of CH (20). This case illustrates further phenotypic heterogeneity associated with human TTF-2 mutations and suggests that defects in this gene should also be considered in cases of syndromic CH with cleft palate, but not necessarily complete thyroid agenesis. This variable phenotype may reflect differential effects of TTF-2 mutations on downstream target genes required for normal human thyroid organogenesis, migration, and differentiation, and the further identification of such genes may elucidate these mechanisms and provide novel genetic candidates for CH and cleft palate.

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